

# Sub-epidemics Explain Localized High Prevalence of Reduced Susceptibility to Rilpivirine in Treatment-Naïve HIV-1-Infected Patients: Subtype and Geographic Compartmentalization of Baseline Resistance Mutations

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## Abstract

**Objective:** The latest nonnucleoside reverse transcriptase inhibitor (NNRTI) rilpivirine (RPV) is indicated for human immunodeficiency virus type-1 (HIV-1) patients initiating antiretroviral treatment, but the extent of genotypic RPV resistance in treatment-naïve patients outside clinical trials is poorly defined.

**Study Design:** This retrospective observational study of clinical data from Belgium and Portugal evaluates genotypic information from HIV-1 drug-naïve patients obtained for the purpose of drug resistance testing. Rilpivirine resistance-associated mutations (RPV-RAMs) were defined based on clinical trials, phenotypic studies, and expert-based resistance algorithms. Viral susceptibility to RPV alone and to the single-tablet regimen was estimated using expert-based resistance algorithms.

**Results:** In 4,631 HIV-1 treatment-naïve patients infected with diverse HIV-1 subtypes, major RPV-RAMs were detected in 4.6%, while complete viral susceptibility to RPV was estimated in 95% of patients. Subtype C- and F1-infected patients displayed the highest levels of reduced viral susceptibility at baseline, respectively 13.2% and 9.3%, mainly due to subtype- and geographic-dependent occurrence of RPV-RAMs E138A and A98G as natural polymorphisms. Strikingly, a founder effect in Portugal resulted in a 138A prevalence of 13.2% in local subtype C-infected treatment-naïve patients. The presence of transmitted drug resistance did not impact our estimates.

**Conclusion:** RPV is the first HIV-1 inhibitor for which, in the absence of transmitted drug resistance, intermediate or high-level genotypic resistance can be detected in treatment-naïve patients. The extent of RPV susceptibility in treatment-naïve patients differs depending on the HIV-1 subtype and dynamics of local compartmentalized epidemics. The highest prevalence of reduced susceptibility was found to be 15.7% in Portuguese subtype C-infected treatment-naïve patients. In this context, even in the absence of transmitted HIV-1 drug resistance (TDR), drug resistance testing at baseline should be considered extremely important before starting treatment with this NNRTI.

## Introduction

**R**ILPIVIRINE (RPV) IS THE latest nonnucleoside reverse transcriptase (RT) inhibitor (NNRTI) approved for antiretroviral treatment (ART) of human immunodeficiency

virus type-1 (HIV-1) infection. RPV is currently indicated for treatment-naïve patients with a viral load lower than 100,000 copies/mL,<sup>1</sup> and predominantly administered first-line as a single-tablet regimen (STR) with a fixed-dose coformulation containing nucleos(t)ide RT

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inhibitors (NRTIs) tenofovir disoproxil fumarate (TDF) and emtricitabine (FTC).

While the phase 3 clinical studies ECHO and THRIVE demonstrated noninferior virological efficacy and safety of RPV compared to efavirenz,<sup>2,3</sup> these clinical studies precluded HIV-1 patients showing NNRTI mutations at baseline. Although a low prevalence of RPV resistance-associated mutations (RAMs) was described outside these clinical trials, RPV-RAMs have been reported to occur naturally as polymorphisms, indicating an impact of HIV-1 subtype on the RPV activity.<sup>4–6</sup> In this study, we evaluate the genotypic resistance profile of RPV in a large database with HIV-1 genetic sequences of treatment-naïve patients infected with different subtypes. Viral susceptibility predictions were used to better understand the clinical benefit of RPV for first-line HIV-1 treatment.

## Materials and Methods

Clinical data of HIV-1 treatment-naïve patients pooled from a large HIV-1 drug resistance database in Portugal ( $n=4,541$ ) and from the University Hospitals Leuven in Belgium ( $n=777$ ), obtained for the purpose of routine genotypic resistance testing before cART initiation, were retrospectively examined.<sup>7,8</sup> The study protocol was conducted in accordance with the Declaration of Helsinki and approved by the ethics committees of UZ Leuven (B322201420270/S56109) and Centro Hospitalar de Lisboa Ocidental (108/CES-2014). For each patient, the first available viral isolate spanning the RT region was collected. HIV-1 subtype was assigned by Rega V3<sup>9</sup> and COMET<sup>10</sup> subtyping tools, and only the most prevalent subtypes concordantly determined by both tools were retained for the final analysis, thereby excluding 687 patients. As a result, the analysis was performed on 4,631 patients (715 from Belgium and 3,916 from Portugal).

Major RPV resistance-associated mutations (RPV-RAMs) were defined based on data from the clinical trials, phenotypic RPV resistance analyses, and package inserts: K101E/P, E138A/G/K/Q/R, V179L, Y181C/I/V, Y188L, H221Y, F227C, M230I/L, and the mutational combination of L100I+K103N.<sup>1,11,12</sup> In addition, we defined a list of minor RPV-RAMs that have been observed in *in vitro* or *in vivo* selection studies and are included in one or more of clinically widely used genotypic resistance interpretation algorithms ANRS (V24), Rega (V9.1.0), and HIVdb (V7.0.1),<sup>1–16</sup> encompassing V90I, A98G, L100I/V, K101H/Q/T, K103R/S, V106A/I, V108I, E138S, V179D/E/F/I/T, Y181F/G/S, Y188F, V189I, G190A/C/E/Q/S/T/V, and M230V.

Clinical implications of observed RPV genotypic resistance were assessed by classifying viral isolates as susceptible, intermediate resistant, or high-level resistant according to Rega and HIVdb, and susceptible or resistant according to ANRS. For HIVdb, five-level scores were simplified to three levels: susceptible and potential low-level resistant were scored as susceptible, low-level and intermediate resistant were scored as intermediate resistant, and high-level resistant as resistant. An estimate of preserved viral susceptibility was obtained by averaging over the three algorithms. The activity of the RPV-containing STR was also assessed by estimating viral susceptibility to NRTIs TDF and FTC.

Evidence of transmitted HIV-1 drug resistance (TDR) was defined by the presence of at least one surveillance drug

resistance mutation (SDRM) from the consensus genotypic definition of Bennett *et al.*,<sup>17</sup> including major RVP-RAMs L100I+K103N, Y181C/I/V, Y188L, and M230L, and minor RVP-RAMs L100I, K103S, V106A, V179F, and G190A/E/S.

The origin of E138A in subtype C-infected treatment-naïve patients was further investigated by phylogenetic analysis and transmission ratio calculation. Specifically, control sequences were selected using two different approaches. First, a BLAST search was performed to identify the 10 most similar sequences to each subtype C-infected treatment-naïve patient included in this study, and their treatment status was retrieved from the original publications. Second, sequence data from treatment-experienced patients infected with subtype C were collected from the Portuguese and Leuven cohorts. HIV-1 subtype was confirmed as described above and duplicate or clonal sequences were removed, resulting in a dataset of 1,121 sequences. SDRM-related positions as well as the E138 codon were excluded from the alignment. For phylogenetic analysis, two separate approaches were used. First, a maximum likelihood tree was built using RaxML, including all 1,121 sequences in the analysis. Second, to evaluate the reproducibility of a monophyletic cluster of sequences containing E138A, 20 maximum likelihood trees with each including 200 sequences randomly selected from this large dataset were constructed using PhyML.<sup>18</sup> The transmission ratio of E138A was calculated by dividing its prevalence in treatment-naïve patients by the prevalence in treatment-experienced patients and interpreted following Winand *et al.*<sup>19</sup>

Data were analyzed using the package R, with a level of significance set at 5% and Benjamini–Hochberg correction for multiple testing.<sup>20</sup>

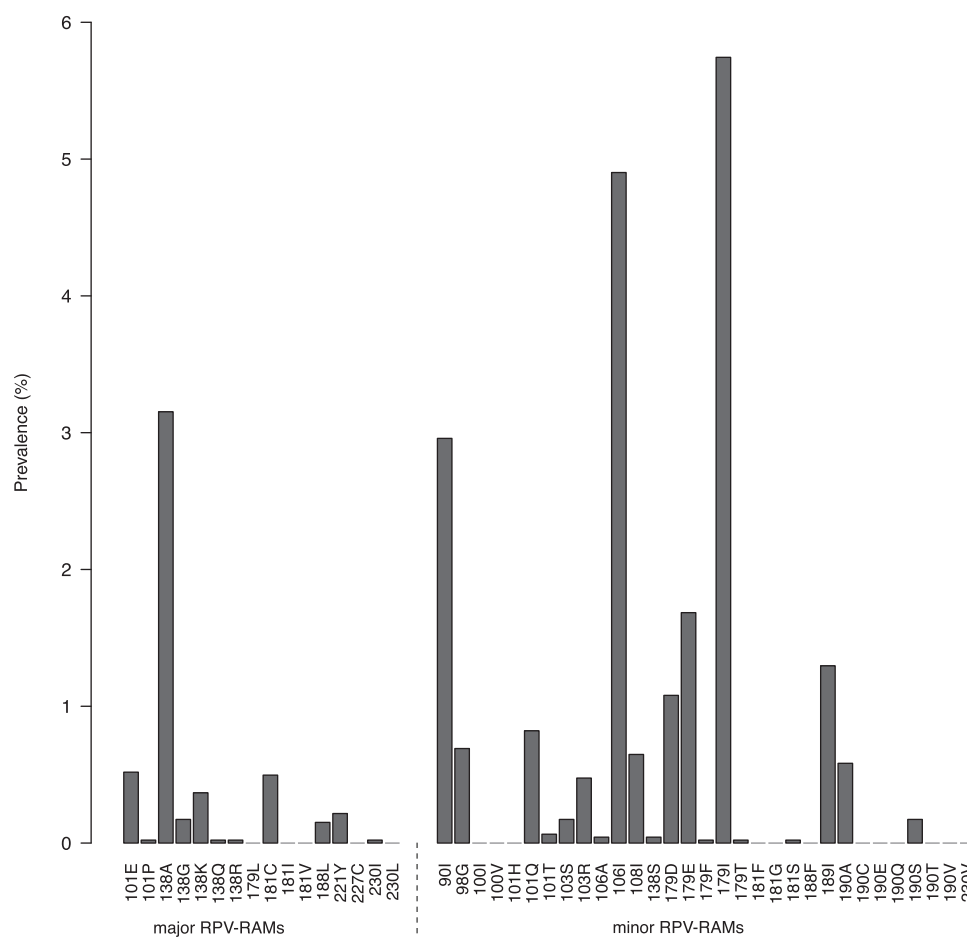
## Results

Genotypic information from 4,631 treatment-naïve patients revealed one or more major RPV-RAMs in 4.6% ( $n=213$ ) of patients, predominantly as a single mutation (4.1%,  $n=189$ ), but also within a combination of two (0.5%,  $n=22$ ) or three major mutations (0.04%,  $n=2$ ). Furthermore, at least one minor RPV-RAM was present in 18.4% ( $n=851$ ) of patients, with one (16.4%,  $n=761$ ), two (1.4%,  $n=64$ ), and three, or more minor mutations (0.6%,  $n=26$ ). In total, 21.5% ( $n=997$ ) of the patients presented at least one major and/or minor RPV mutation. Figure 1 shows the prevalence of individual RPV-RAMs. The most prevalent major RPV-RAM was E138A (3.2%,  $n=146$ ), occurring in 69% of all patients with  $\geq 1$  major RPV-RAM, followed by K101E (0.5%,  $n=24$ ). Minor RPV-RAMs V179I (5.7%,  $n=266$ ), V106I (4.9%,  $n=227$ ), and V90I (3.0%,  $n=137$ ) occurred most. Major signature NRTI mutations against TDF and FTC M184V (0.8%,  $n=35$ ), M184I (0.07%,  $n=3$ ), K65R (0.07%,  $n=3$ ), and K70E (0.04%,  $n=2$ ) were rarely observed.

When averaged over the three interpretation systems, viral susceptibility to the STR and RPV was predicted as preserved in  $94.0\% \pm 1.0\%$  and  $95.0\% \pm 0.7\%$  of the patients, respectively, indicating that loss in viral susceptibility to the STR was largely attributable to RPV resistance.

An impact of HIV-1 natural diversity was evaluated by comparing the distribution of RPV-RAMs and the predicted activity across HIV-1 subtypes and CRFs (Table 1). Patients were predominantly infected with HIV-1 subtype B ( $n=2,110$ ,

**FIG. 1.** Prevalence of individual major (*left*) and minor (*right*) rilpivirine resistance-associated mutations (RPV-RAMs).



45.6%) and subtype G ( $n=1,345$ , 29.0%), followed by CRF 02\_AG ( $n=451$ , 9.7%), subtype C ( $n=345$ , 7.5%), A1 ( $n=194$ , 4.2%), F1 ( $n=132$ , 2.9%), and CRF 01\_AE ( $n=54$ , 1.2%). The prevalence of  $\geq 1$  major or  $\geq 1$  minor RPV-RAMs was significantly different across subtypes (chi-square test,  $p < .001$ ). The highest prevalence of major RPV-RAMs was observed for subtypes C (12.5%) and F1 (6.8%), largely explained by the subtype-specific occurrence of E138A with, respectively, 11.3% and 6.1% (Table 1). A variable prevalence of minor RPV-RAMs was observed, with minor RPV-RAMs V90I, A98G, V106I, and V179D/E/I/T differing significantly across subtypes, when corrected for multiple testing. A discrepancy in the proportion of patients scored susceptible to RPV was also observed (Table 1). Viral susceptibility was particularly reduced in patients infected with subtypes C or F1, respectively, only  $86.8\% \pm 0.8\%$  and  $90.7\% \pm 4.4\%$  of patients scored susceptible to RPV.

RPV susceptibility in subtype C-infected patients differed according to geographic origin of sampling, with  $84.3\% \pm 1.1\%$  of patients from Portugal who scored susceptible compared to  $95.0\% \pm 0.0\%$  of patients from Belgium (Table 1), which was largely explained by a prevalence of RPV-RAM E138A of, respectively, 13.2% and 5.0% ( $p = .044$ ). Subtype F1-infected patients displayed a lower proportion that was estimated susceptible (90.7%) together with high between-algorithm variability (4.4%). A higher prevalence of E138A was also observed in subtype F1-infected patients from Portugal (7.1%), explaining that low proportion of patients scored susceptible

(92.0%  $\pm$  0.0%). Subtype F1-infected patients from Belgium however lacked E138A, but they still displayed a lower average susceptibility score ( $82.5\% \pm 30.1\%$ ) mainly attributable to the minor RVP-RAM A98G (52.6%). This minor RPV-RAM is however only scored by the HIVdb algorithm, but not by ANRS or Rega, with only 47.4% of patients estimated susceptible by HIVdb.

Evidence of transmitted drug resistance was observed in 401 patients (8.7%), with RPV-RAMs Y181C, Y188L, K103S, V106A, V179F, and G190A/S detected as SDRMs. When excluding these patients, one or more major RPV-RAMs could still be detected in 3.4% of the remaining 4,230 study patients and at least one minor RPV-RAM in 16.6%, while preserved susceptibility to RPV remained in  $96.0\% \pm 1.0\%$  of patients. The frequency of major RPV-RAM E138A in this population was 3.1% compared to 4.2% in the patient population with SDRMs, although strongly varying according to geography and subtype (Table 2). Strikingly, among 116 subtype C-infected treatment-experienced patients from Portugal, the RPV-RAM E138A could be detected in 11 patients (9.5%), compared to 35 treatment-naïve patients (13.2%), resulting into a transmission ratio of 1.39.

To investigate whether a founder effect contributed to the propagation of this polymorphism, a set of 1,121 subtype C sequences was compiled, which included the most similar subtype C sequences, determined as described in the Materials and Methods section. Phylogenetic analysis of the 1,121 subtype C-infected patient population revealed that a large

TABLE 1. FREQUENCY OF RILPIVIRINE MUTATIONS ACROSS SUBTYPES

	n	%	<i>AI</i>	<i>B</i>	<i>C</i>	<i>FI</i>	<i>G</i>	<i>OI_AE</i>	<i>O2_AG</i>
Patients									
All	4,631	100	4.2	45.6	7.5	2.9	29.0	1.2	9.7
Portugal	3,916	100	3.5	43.6	6.8	2.9	33.9	0.1	9.3
Belgium	715	100	7.8	56.8	11.2	2.7	2.2	7.1	12.2
≥1 major									
All	213	4.6	3.1	4.4	12.5	6.8	3.1	1.9	4.2
Portugal	187	4.8	3.6	4.4	14.7	8.0	3.2	0.0	4.7
Belgium	26	3.6	1.8	4.4	5.0	0.0	0.0	2.3	2.0
E138A									
All	146	3.2	3.1	2.8	11.3	6.1	1.8	1.8	1.8
Portugal	124	3.2	3.6	2.6	13.2	7.1	1.8	0.0	2.2
Belgium	22	3.1	1.8	3.9	5.0	0.0	0.0	2.0	0.0
≥1 minor									
All	851	18.4	62.4	20.1	8.4	29.5	10.6	33.3	1.8
Portugal	686	17.5	63.0	20.2	10.5	25.6	10.3	66.7	15.9
Belgium	165	23.0	60.7	19.4	1.3	52.6	37.5	31.3	21.8
V90I									
All	137	3.0	1.5	4.1	0.3	1.5	0.8	0.0	7.3
Portugal	101	2.6	1.5	3.8	0.4	1.8	0.7	0.0	6.0
Belgium	36	5.0	1.8	5.4	0.0	0.0	12.5	0.0	12.6
A98G									
All	32	0.7	0.5	0.6	1.7	7.6	0.1	0.0	0.4
Portugal	22	0.6	0.7	0.7	2.3	0.0	0.1	0.0	0.5
Belgium	10	1.4	0.0	0.0	0.0	52.6	0.0	0.0	0.0
V106I									
All	227	4.9	1.0	7.6	0.0	5.3	3.3	7.4	1.8
Portugal	207	5.3	0.0	8.6	0.0	6.2	3.4	0.0	1.9
Belgium	20	2.8	0.0	3.7	0.0	0.0	0.0	7.8	1.1
V179I									
All	266	5.7	59.3	4.0	3.2	13.6	0.7	18.5	4.0
Portugal	192	4.9	60.1	3.5	4.2	15.9	0.7	33.4	3.0
Belgium	74	10.4	57.1	6.4	0.0	0.0	0.0	17.6	8.0
V179D									
All	50	1.1	1.0	1.9	0.6	2.3	0.1	3.7	0.0
Portugal	39	1.0	0.7	1.9	0.8	2.7	0.0	0.0	0.0
Belgium	11	1.5	1.8	1.7	0.0	0.0	6.3	3.9	0.0
V179E									
All	78	1.7	0.5	1.0	0.3	0	3.6	1.9	1.1
Portugal	75	1.9	0.7	1.1	0.4	0.0	3.7	0.0	1.4
Belgium	3	0.4	0.0	0.5	0.0	0.0	0.0	2.0	0.0
V179T									
All	1	0.0	0.0	0.0	0.0	0.8	0.0	0.0	0.0
Portugal	1	0.0	0.0	0.0	0.0	0.9	0.0	0.0	0.0
Belgium	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Active RPV									
Mean ± SD									
All	—	95.0±0.7	96.6±0.6	95.1±0.7	86.8±0.8	90.7±4.4	96.8±0.2	98.1±0.0	95.4±0.3
Portugal	—	94.8±0.5	96.1±0.4	95.1±0.8	84.3±1.1	92.0±0.0	96.7±0.2	100±0.0	94.9±0.3
Belgium	—	95.7±1.0	97.6±1.0	95.4±0.3	95.0±0.0	82.5±30.1	100±0.0	98.0±0.0	97.7±0.0
ANRS									
All	4,416	95.4	96.9	95.6	87.2	93.2	96.9	98.1	95.6
Portugal	3,727	95.2	96.4	95.6	84.9	92.0	96.8	100	95.1
Belgium	689	96.3	98.2	95.6	95.0	100	100	98.0	97.7
HIVdb									
All	4,366	94.3	95.8	94.3	85.8	85.6	96.6	98.1	95.1
Portugal	3,690	94.2	95.7	94.1	83.0	92.0	96.6	100	94.5
Belgium	676	94.5	96.4	95.1	95.1	47.4	100	98.0	97.7
Rega									
All	4,413	95.3	96.9	95.5	87.2	93.2	96.9	98.1	95.6
Portugal	3,724	95.1	96.4	95.4	84.9	92.0	96.8	100	95.1
Belgium	689	96.4	98.2	95.6	95.0	100	100	98.0	97.7

The distribution of HIV-1 subtypes among the 4,631 treatment-naïve patients is indicated, with for each subtype, the proportion (%) of patients displaying ≥1 major RPV-RAMs, ≥1 minor RPV-RAMs, single RPV-RAMs, and predicted activity to RPV.

Single mutations were only listed when their prevalence significantly differed across subtypes. Estimated drug activity is shown for each individual genotypic resistance interpretation algorithm (ANRS V24, Rega V9.1.0 and HIVdb V7.0.1) and the mean of estimated proportion of each resistance interpretation algorithm together with the SD.

HIV-1, human immunodeficiency virus type-1; RPV-RAMs, rilpivirine resistance-associated mutations; SD, standard deviation.

TABLE 2. FREQUENCY OF E138A AND TRANSMITTED DRUG RESISTANCE

Subtype	Belgium (n=715)			Portugal (n=3,916)		
	TDR (%)	E138A (%)		TDR (%)	E138A (%)	
		WT	TDR		WT	TDR
All	9.9	3.3	1.4	8.4	3.0	4.9
A1	1.8	1.8	0	3.6	3.8	0.0
B	14.5	4.3	1.7	10.9	2.8	0.6
C	3.8	5.2	0.0	6.0	11.7	37.5
F1	5.3	0.0	0.0	5.3	7.5	0.0
G	6.3	0.0	0.0	6.9	1.3	8.8
AE	3.9	2.0	0.0	0.0	0.0	0.0
AG	4.6	0.0	0.0	7.1	2.1	3.9

The frequency of TDR and of RPV-RAM E138A in patients with (TDR) or without SDRMs (WT), according to subtype and geographical origin of the patient (Belgium–Leuven Cohort, Portugal, Portuguese Cohort).

SDRM, surveillance drug resistance mutation; TDR, transmitted HIV-1 drug resistance; WT, wild-type.

number of Portuguese treatment-naïve patients with E138A formed a monophyletic cluster (Supplementary Fig. S1; Supplementary Data are available online at [www.liebertpub.com/aid](http://www.liebertpub.com/aid)). Since this cluster was not supported by a large bootstrap support, we used subsampling to evaluate the reproducibility of this cluster. Twenty separate phylogenetic analyses, each containing a subsample of 200 patients of the initial dataset, demonstrated a consistent monophyletic grouping of this E138A cluster among all 20 ML trees (100%), with an aLRT support for this cluster above 0.8 in 16 trees (80%) (Data not shown).

## Discussion

The latest NNRTI RPV is an attractive first-line option for treatment of HIV-1 infection due to its favorable tolerability and dosing, but its use should be guided by resistance testing at baseline as for other antiviral drugs, especially NNRTIs.<sup>1,21</sup> Using a large population of treatment-naïve HIV-1 patients, we report reduced RPV susceptibility at baseline estimated in ~5% of HIV-1 treatment-naïve patients. However, variable prevalence of RPV-RAMs was detected when HIV-1 subtypes were evaluated separately, with subtypes C and F1 showing a higher prevalence of reduced baseline susceptibility to RPV.

Specifically, subtype C treatment-naïve patients in our datasets displayed an increased prevalence of RPV-RAM E138A, which when present alone confers intermediate or high-level resistance by all three algorithms. This natural polymorphism is known to occur in varying frequencies according to viral subtype and is previously shown to have a higher prevalence in subtype C patients compared to subtype B patients.<sup>6</sup> In our study, subtype F1 strains circulating in the Portuguese HIV-1 epidemic also showed an increased prevalence of this polymorphism, while subtype F1 patients in Belgium displayed an increased prevalence of mutation A98G. Importantly, the RPV-RAM A98G is only assigned an intermediate resistance score for RPV by the HIVdb algorithm and is not considered by the ANRS and Rega algorithms.

The findings of our study are in large agreement with a similar study by Lambert-Niclot *et al.* that observed a prevalence of 4.6% for major RPV-RAMs and 19.9% for any RPV mutation in 1,729 drug-naïve patients, of which 4.9% were scored resistant by ANRS.<sup>4</sup> They also reported a higher prevalence of RPV genotypic resistance in pooled non-B

subtype-infected patients compared to subtype B-infected patients, without a stratification by subtypes, however. Furthermore, trends in RPV-RAMs prevalence across subtypes observed in our study were highly comparable with mutation frequencies according to subtype reported by the Stanford HIV Drug Resistance Database.<sup>22</sup>

Importantly, our study shows that the prevalence of E138A and A98G is highly determined by geographical compartmentalization of subtype epidemics, with a higher prevalence of E138A in subtype C and F1 patients from Portugal and a higher prevalence of A98G in subtype F1 patients from Belgium. These observations suggest founder effects boosting local transmission of these mutations, an HLA impact for the selection of these mutations in each population, and/or differences in selective pressure caused by different treatment strategies between countries.<sup>23</sup>

Phylogenetic analysis of subtype C sequences supported the hypothesis that a founder effect in the Portuguese treatment-naïve patients explains the high prevalence of E138A in Portugal. Furthermore, a transmission ratio of 1.39 was detected for E138A, indicating that high levels of transmission between treatment-naïve patients contribute to its higher prevalence. This finding suggests that the origin of E138A in Portugal is most probably not the treated population and confirms its forward transmission among treatment-naïve patients.<sup>19</sup> Despite its reported selection by ART, the E138A mutation is not included in the surveillance mutation list of Bennett *et al.* due to the polymorphic nature of RT position 138.<sup>17</sup> A prevalent E138A in treatment-naïve patients could have accompanied transmitted DRMs, however, our results strongly suggest that the reduced viral susceptibility to RPV and in particular the increased E138A prevalence in a geographic and subtype-dependent manner resulted from the propagation of a natural polymorphism following a founder event.

Regarding the origin of an increased E138A presence in treatment-naïve patients, a distinction between original transmission from treated patients and increased prevalence of a natural polymorphism after a founder effect can have important implications for the treatment of HIV-1 infected patients. If E138A predominantly results from transmission from treated patients, its high prevalence would largely coincide with regions with substantial ART coverage and could be addressed by targeted intervention strategies or optimized treatment

policies in addition to resistance testing that is usually recommended before ART initiation in these regions.

This study, however, finds that natural variability and founder effects can boost the natural occurrence of E138A, suggesting that even regions with low ART coverage can display a high prevalence of RPV resistance, with potentially devastating implications when RPV-based first-line treatment is initiated in the absence of routine resistance testing of treatment-naïve patients.<sup>1</sup> In addition to supporting large-scale analyses of RPV genotypic resistance in treatment-naïve patients,<sup>22</sup> the significance of our findings should also be further elucidated in localized epidemics characterized by similar and other subtypes.

In this study, we show a high prevalence of reduced susceptibility to RPV at baseline in all subtypes, but with a significant variability both between HIV-1 subtypes and between local epidemics. These findings have an important impact for the use of RPV as first-line treatment. Loss of RPV activity directly affects the success of this low genetic barrier drug combination, but also limits future treatment options due to extensive cross-resistance to other NNRTIs and most likely also to other NRTIs upon failure of this regimen. In the context of such a high prevalence of reduced susceptibility to RPV at baseline, even in patients without TDR, drug resistance testing at baseline should be considered extremely important before starting treatment with this NNRTI.

Furthermore, the fact that the prevalence of RPV-RAMs differed between local subtype epidemics indicates a geographical compartmentalization of such variants that should be further investigated and monitored. Finally, the finding of discrepancies between drug resistance algorithms indicates the need for a better consensus on the impact of RPV-RAMs. In summary, our results indicate that drug resistance to RPV should be more thoroughly investigated. Until then, drug resistance testing at baseline is crucial for patients initiating therapy with an RPV containing regimen.

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## Sequence Data

We acknowledge the ARHR policy about data availability. We have not provided the accession numbers for the dataset because these sequences are not deposited in GenBank as they comprise a dense sampling of one region. They have been deposited in Euresist ([www.euresist.org](http://www.euresist.org)), a project aiming to collect and make available data to study HIV drug resistance and viral diversity in Europe. Access to the sequences linked to all clinical and demographic data is available through Euresist.

## Author Disclosure Statement

No competing financial interests exist.

## References

1. EDURANT® (rilpivirine) package insert. Available at [www.ema.europa.eu/docs/en\\_GB/document\\_library/EPAR\\_-\\_Product\\_Information/human/002264/WC500118874.pdf](http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/002264/WC500118874.pdf), accessed March 12, 2014.
2. Molina J-M, Cahn P, Grinsztejn B, *et al.*: For ECHO Study Group: Rilpivirine versus efavirenz with tenofovir and emtricitabine in treatment-naïve adults infected with HIV-1 (ECHO): A phase 3 randomised double-blind active-controlled trial. *Lancet* 2011;378:238–246.
3. Cohen CJ, Andrade-Villanueva J, Clotet B, *et al.*: For THRIVE Study Group: Rilpivirine versus efavirenz with two background nucleoside or nucleotide reverse transcriptase inhibitors in treatment-naïve adults infected with HIV (THRIVE): A phase 3, randomised, non-inferiority trial. *Lancet* 2011;378:229–237.
4. Lambert-Niclot S, Charpentier C, Storto A, *et al.*: Prevalence of pre-existing resistance-associated mutations to rilpivirine, emtricitabine and tenofovir in antiretroviral-naïve patients infected with B and non-B subtype HIV-1 viruses. *J Antimicrob Chemother* 2013;68:1237–1242.
5. Sungkanuparph S, Jiamsakul A, Kiertiburanakul S, *et al.*: TREAT Asia studies to evaluate resistance: Rilpivirine resistance-associated mutations among antiretroviral-naïve patients infected with HIV-1 in Asia. *J Acquir Immune Defic Syndr* 2013;62:e98–e100.
6. Sluis-Cremer N, Jordan MR, Huber K, *et al.*: E138A in HIV-1 reverse transcriptase is more common in subtype C than B: Implications for rilpivirine use in resource-limited settings. *Antiviral Res* 2014;107:31–34.
7. Theys K, Vercauteren J, Abecasis AB, *et al.*: The rise and fall of K65R in a Portuguese HIV-1 Drug Resistance database, despite continuously increasing use of tenofovir. *Infect Genet Evol* 2009;9:683–688.
8. Libin P, Beheydt G, Deforche K, *et al.*: RegaDB: Community-driven data management and analysis for infectious diseases. *Bioinformatics* 2013;29:1477–1480.
9. Pineda-Peña AC, Faria NR, Imbrechts S, *et al.*: Automated subtyping of HIV-1 genetic sequences for clinical and surveillance purposes: Performance evaluation of the new REGA version 3 and seven other tools. *Infect Genet Evol* 2013;19:337–348.
10. Struck D, Lawyer G, Ternes AM, Schmit JC and Bercoff, DP: COMET: Adaptive context-based modeling for ultra-fast HIV-1 subtype identification. *Nucleic Acids Res* 2014; 18:e144.
11. Vingerhoets J, Rimsky L, Van Eygen V, *et al.*: Pre-existing mutations in the rilpivirine Phase III trials ECHO and

- THRIVE: Prevalence and impact on virologic response. *Antivir Ther* 2013;18:253–256.
12. Rimsky L, Vingerhoets J, Van Eygen V, *et al.*: Genotypic and phenotypic characterization of HIV-1 isolates obtained from patients on rilpivirine therapy experiencing virologic failure in the phase 3 ECHO and THRIVE studies: 48-week analysis. *J Acquir Immune Defic Syndr* 2012;59:39–46.
  13. Azijn H, Tirry I, Vingerhoets J, *et al.*: TMC278, a next-generation nonnucleoside reverse transcriptase inhibitor (NNRTI), active against wild-type and NNRTI-resistant HIV-1. *Antimicrob Agents Chemother* 2010;54:718–727.
  14. Meynard JL, Vray M, Morand-Joubert L, *et al.*: Phenotypic or genotypic resistance testing for choosing antiretroviral therapy after treatment failure: A randomized trial. *AIDS* 2002;16:727–736.
  15. Liu TF, Shafer RW: Web resources for HIV type 1 genotypic-resistance test interpretation. *Clin Infect Dis* 2006;42:1608–1618.
  16. Vercauteren J, Beheydt G, Prosperi M, *et al.*: Clinical evaluation of Rega 8: An updated genotypic interpretation system that significantly predicts HIV-therapy response. *PLoS One* 2013;8:e61436.
  17. Bennett DE, Myatt M, Bertagnolio S, Sutherland D, Gilks CF: Recommendations for surveillance of transmitted HIV drug resistance in countries scaling up antiretroviral treatment. *Antivir Ther* 2008;2:25–36.
  18. Guindon S, Dufayard JF, Lefort V, *et al.*: New algorithms and methods to estimate maximum-likelihood phylogenies: Assessing the performance of PhyML 3.0. *Syst Biol* 2010;59:307–321.
  19. Winand R, Theys K, Eusebio M, *et al.*: Assessing transmissibility of HIV-1 drug resistance mutations from treated and from drug-naïve individuals. *AIDS* 2015;29:2045–2052.
  20. R Development Core Team: R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, 2011.
  21. Wittkop L, Günthard HF, de Wolf F, *et al.*: Effect of transmitted drug resistance on virological and immunological response to initial combination antiretroviral therapy for HIV (EuroCoord-CHAIN joint project): A European Multicohort Study. *Lancet Infect Dis* 2011;5:363–371.
  22. Rhee SY, Kantor R, Katzenstein DA, *et al.*: HIV-1 pol mutation frequency by subtype and treatment experience: Extension of the HIVseq program to seven non-B subtypes. *AIDS* 2006;20:643–651.
  23. Gatanaga H, Murakoshi H, Hachiya A, *et al.*: Naturally selected rilpivirine-resistant HIV-1 variants by host cellular immunity. *Clin Infect Dis* 2013;7:1051–1055.

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